

Evolutionary Ecology of Bacterial Viruses

Linking host-phage data with analysis at multiple scales could lead to refinement of a theoretical foundation for microbial ecology

Joshua S. Weitz

Host-phage interactions provide a tractable model system for linking data and theory at multiple scales and could help in developing a new theoretical foundation for microbial ecology. This relatively simple system could prove to be a benchmark for microbiologists who are probing more complex cellular and ecosystem processes with increasingly finer resolution, producing an expanding surplus of data through a varied means of powerful experimental techniques. Recently, applications of mathematical concepts to microbiology have found successes in efforts to organize and reduce the complexity of high-throughput data. Of course, relying on quantitative methods to parse data cannot replace the hard thinking and additional theoretical tools needed to uncover meaningful biological insights.

Dealing with complex data sets is but one step of a larger analytic process (Fig. 1). For example, a multisequence alignment program may

generate a plausible phylogenetic tree from a set of genomic sequences. However, interpreting the structure of that tree also may depend on applying another theory at the level of strain evolution and epidemiological dynamics. More generally, theory exists at multiple scales, from improved methods of dimensional reduction to theories of biological dynamics to *ab initio* methods meant to simulate a cell from the bottom up. What distinguishes this new age of theory from previous incursions into biology is that so much of it aims to connect macroscopic properties to those at microscopic levels with unprecedented detail.

Thus far, theoretical analyses largely focus on the first part of the chain, such as dimensional reduction and representation, despite recent efforts to develop general principles for microbial ecology (see *Microbe*, April 2006, p. 175). This trend is unsurprising because the rate at which data are being generated demands new and innovative ways to represent, classify, and compare genomes, sequence fragments, gene expression, enzymatic activity, and extracellular signaling.

However, there is much to care about at the other end of that chain, where quantitative predictions are sought concerning how some perturbation will change the functioning of a microbial and viral community. For example, if a bioremediating microbe is introduced, how is it affected by the population abundance of competitors? Or, what is the most likely species for a cross-species jump of a more virulent form of a zoonotic pathogen? The answers to such questions might depend on details at the genomic-sequence or biochemical level, or just as importantly, the state of biological populations and the dynamics of how they mix.

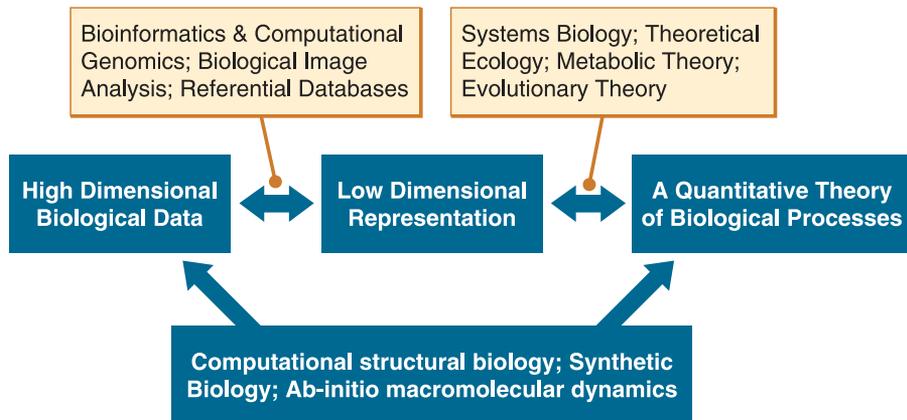
Summary

- Phages, long studied as model systems in molecular biology, are increasingly recognized as key components of ecosystems.
- Environmental phages affect global nutrient cycling, the diversity of bacteria, and the course of infectious diseases.
- Interactions between hosts and phages provide a means for linking systems biology and evolutionary ecology.
- To improve our understanding of the evolution and ecology of phages, we will need to develop new techniques for scaling up microscopic dynamics in the face of changing biological components.

Joshua S. Weitz is an Assistant Professor in the School of Biology, Georgia Institute of Technology, Atlanta.



FIGURE 1



Theoretical studies in microbiology link data at multiples scales. Major research avenues include forging links between high and low dimensional representations of data and biological process.

Bacterial Viruses in Ecology

Bacteriophages, the most abundant organisms on the planet, are amazingly diverse. They shuttle genes, modify population dynamics of their hosts, alter host metabolism of marine autotrophs, influence the spread of infectious diseases, and impinge on global nutrient recycling and biogeochemical cycles. Yet, in the 80 years since their discovery, phages were studied nearly exclusively, albeit productively, as model systems in molecular biology.

Any attempt at accounting for the ecological impact of phages requires us to consider their behavior and dynamics at multiple scales. Imagine following the life cycle of a phage. At each stage, the life history of a phage is intertwined with major challenges in systems biology, microbiology, and evolutionary ecology (Fig. 2). Key examples include relationships between binding specificity and strain diversification, the mechanisms of regulatory switches, the rate and extent of horizontal gene transfer, and the cycling of nutrients on a global scale.

Much of what we know about how bacterial viruses exploit hosts comes from studying a few types of phages, e.g., λ and the T families, using *Escherichia coli* as a host. Phages have variable host ranges and kinetics that govern how they interact with their bacterial hosts, differing by characteristics such as latent period, burst size, and virion size. In addition, temperate phages vary in terms of the degree to which they pref-

erentially kill or integrate into hosts, their relative rate of spontaneous induction, and the set of genes activated once the phage integrates. The variation of life history traits among environmental phages far exceeds our limited investigations of a few model organisms.

The study of phages also opens the door to questions of decision making in the microbial and viral world. When λ phage infects a bacterial host cell, competition for commonly shared promoters determines whether genes within the lytic or lysogenic pathways are preferentially transcribed. For λ , the likelihood of entering the lysogenic pathway depends on

cell state and the multiplicity of infection. Similarly, the ability of a prophage to induce in response to changes in cellular state determines its relative stability. DNA damage can degrade repressor molecules that maintain the lysogenic state, inducing prophage escape. The plasticity of these switches appears to be highly variable and depends upon ecological context. There is no reason to think that individual phages respond identically to multiplicity of infection, cell stress, or DNA damage.

Single-Scale Models of Dynamic Virion Production

Virion production depends on at least four processes, namely replication of viral genetic material, production of viral structural proteins, insertion of newly made viral genetic material into capsids, and particle release from cells. Multiple studies have shown that overall rates of virion production slow with cell metabolic activity and that viruses can alter the timing of their release based on changes in cellular state or coinfection. Other life history traits are now recognized as being subject to evolutionary and physiological variation.

Recent experimental assays of over a dozen strains of phages suggest a strong, biophysically grounded tradeoff between the extracellular decay rate of virions and the energy invested in each virion. Such a tradeoff is a common reproductive phenomenon in higher organisms. Thus

Weitz Spends Time Being a Phage Biologist, Physicist, Cook, Poet, and Mensch

Joshua Weitz, who trained as a physicist and considers himself a mathematical biologist, at age 10 joined the binary numbers club in elementary school. “It was a very exclusive club,” he says. “We were led to believe that only we and the teacher knew all about these special numbers that made computers work.” As a teenager, he was reading books by and about Albert Einstein, Richard Feynman, and Stephen Hawking, trying to find “the answer to the origins of the universe.”

“It wasn’t until my senior year in college that I began to consider there might be a way to both be a physicist and not be a physicist all at once,” Weitz says, describing his path into biology. “I began to grow tired of the monastic aspect of physics departments, and have since felt much more at home in biology departments. The community tends to be more social, and the problems seem both more relevant and just as fundamental.”

After an influential Santa Fe Institute summer course in complex systems, Weitz switched to biology during his second year in graduate school. “I’ve never regretted the decision,” he says. He still engages in physics, however, noting: “I imagine I will always maintain a somewhat dual approach to the way I practice science.” Weitz is an assistant professor in the school of biology and an adjunct assistant professor in physics at the Georgia Institute of Technology in Atlanta, where his research focuses on bacteriophages.

“Despite being present in nearly every environment where life is

known to exist, we know incredibly little about what phages do, what genes they carry, how they evolve, and how important they are in shaping everything from global nutrient cycles to the dynamics of human disease,” he says. “I am interested in understanding the basic mechanisms by which a phage exploits a host cell, and how these details end up influencing the structure of microbial and viral communities.”

There are practical reasons for studying phages. “If we hope to understand the global cycling of nutrients, then we need to better understand the role of phages in killing key microbial players,” he says. Also, “phages and phage-derived enzymes are increasingly being considered as antibiotic alternatives. However, just as with traditional antibiotics, the evolution of resistance remains a distinct possibility. Finally, I think phages are a great system for theoretical ecologists to consider. It’s a system where evolution is taking place all the time, and to understand the dynamics of phages one has to combine population and evolutionary dynamics.”

Weitz, 32, graduated in 1997 from Princeton University and received his Ph.D from the Massachusetts Institute of Technology (MIT) in 2003. “I was lucky enough to work with an adviser, Daniel Rothman at MIT, who was making a transition toward research at the interface of the biological and physical sciences,” he says. “Dan actively encouraged his students to develop interdisciplinary projects, and this was just fine by me. At first, this meant some misdirection and false

starts, but slowly, some very interesting problems emerged. . . . Uniting these projects was a common interest in developing mathematical models of how biological structures and communities change with time, space, and organismal size. But really, I spent my time exploring, and I wouldn’t have done it any other way.”

Weitz, unmarried but involved in a long-term relationship, writes poetry and likes to cook. “My first dish was stir-fried chicken with lemon sauce, cooked over an electric burner in a kibbutz dorm in Israel during college,” he says. “I’ve written poetry since I was young, and took it up with a greater sense of care and attention to craft when I was in college.” His first book of poetry, *Between Two Stones*, was published by Sheep Meadow Press in 2002. “The book is available online, but old poems, like old scientific papers, seem distant,” he says. He is more excited about his second manuscript, excerpts of which have appeared in small journals, including *Salamander* and the *Nassau Literary Review*.

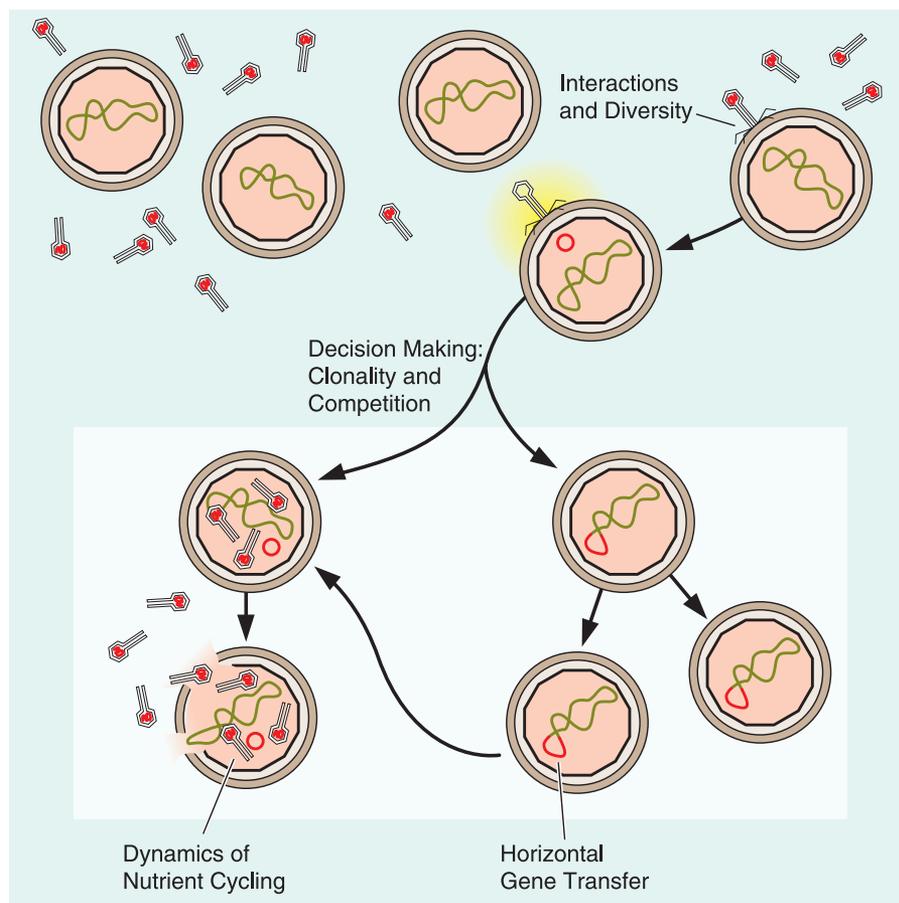
Weitz’s family moved from Washington, D.C., where he was born, to nearby Kensington, Md., in 1980. His father is a lawyer and his mother, a speech pathologist. One of his two sisters is a political scientist and the other is an elementary school teacher. “Without ever talking about it, all three of us ended up becoming educators,” he says. He sees himself striving, as his parents and grandparents urged, “to be a mensch.”

Marlene Cimons

Marlene Cimons is a freelance writer in Bethesda, Md.



FIGURE 2



Life history of viruses from infection to decision-making to dynamics, including possible integration of viral prophage into the host chromosome.

some viral strains produce many offspring but invest little in each, whereas other strains produce very few progeny but invest more in each one. Mathematical models to describe this reproductive tradeoff can be integrated with ecological dynamics of virulent phages at the between-host scale. There is an opportunity here for both theorists and empiricists—as the history of modeling phages reflects the success of researchers working at a single scale, with very few attempts to connect across multiple scales.

The first mathematical expressions to account for host-phage dynamics at the between-host scale were developed in the late 1970s. Phage are obligate intracellular parasites that reproduce within their hosts, whether horizontally as virions or vertically as prophages (Fig. 2). The original mathematical model described the dy-

namics among resources, hosts, and virulent phages in a chemostat with an additional time delay between adsorption and lysis. The key interactions in the Levin-Stewart-Chao model are (i) the growth of hosts on resources and (ii) the phage-induced mortality of hosts with subsequent release of new virions. Rates in this model are assumed to follow linear or Michaelis-Menten kinetics.

The dynamics of such an idealized model mirror, in many respects, those of predator-prey dynamics. For instance, if lysis rates are sufficiently high, hosts and viruses may coexist at the steady state. In experiments, host-phage dynamics recapitulate predictions from theoretical ecology, including what is known as the paradox of enrichment—the case in which increasing resources tend to decrease stability. Newer models incorporate host state as a factor in determining lysis, which suggests another opportunity to link between-host models with biophysically grounded models of exploitation tradeoffs within hosts.

Other models help to address questions of how and why a phage can be temperate. For example, lysogeny enables a phage to avoid extinction when hosts are rare. It also renders hosts immune to lysis by conspecifics—a feature that may benefit both host and phage. A secondary effect is that a subpopulation of lysogens can lyse competing, nearby strains. Rarely have models of temperate phages addressed the adaptive response of phages to host state or embedded these life history strategies in a game theory framework.

Multiscale Models of the Population and Evolutionary Dynamics of Phages

Recently, modelers have begun to turn their attention from the laboratory to the field, and consider the dynamics of phages in complex environments, from microbiomes, to soil, to the world's oceans. Based on empirical findings,

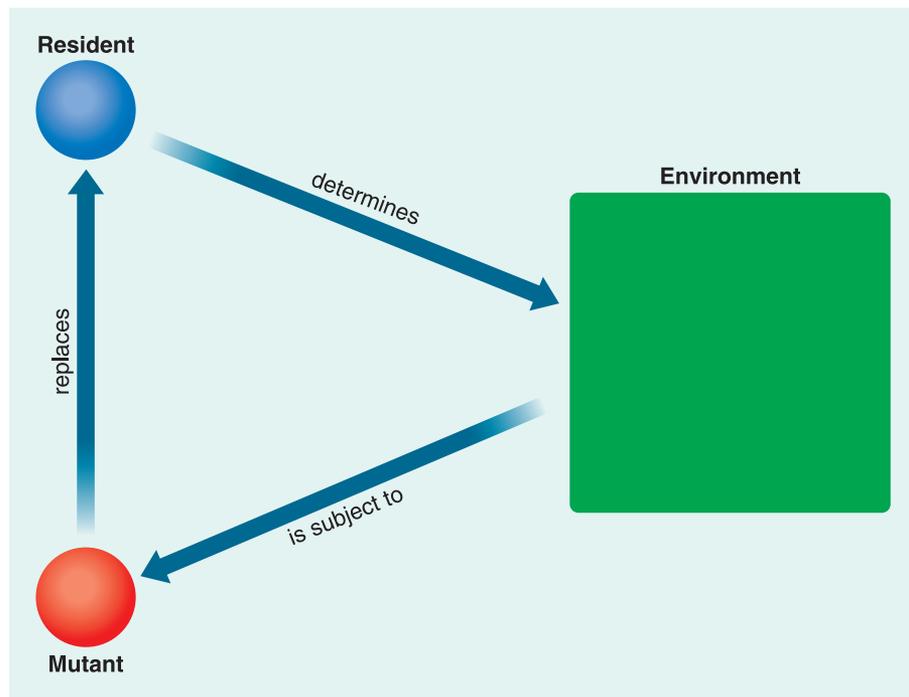
phage densities are estimated to be 10 times greater than their bacterial hosts, with roughly 10–50% of host mortality due to phage lysis. Whether such consensus estimates are due to physiological constraints or environmental factors remains an open question, as is the consistency of the empirical estimates themselves. In order to be relevant, models must be developed that contend with complex biological processes occurring at multiple scales.

Modeling the complexity of environmental phages is particularly difficult, since the evolution of bacterial viruses is intimately linked to their hosts. Phages have substantial variation in burst sizes, extracellular deactivation rates, and adsorption rates. Although the timing of lysis sometimes appears to optimize burst size, the coevolution of phage with their hosts makes it difficult to predict how other phage traits should respond to selective pressures.

Experiments from the 1980s suggest that one-step resistant host mutants could appear rapidly, leading phage to become extinct. However, such scenarios do not describe what happens in even slightly complex environments. For example phages and hosts can diversify rapidly, apparently reflecting linked changes between host growth rates and phage adsorption rates. Other changes, including a phenomenon called “kill the winner,” suggest that virulent phages play an important role in shaping the diversity of their bacterial hosts.

One approach to modeling how phages exploit their hosts is to describe their interaction in terms of game theory instead of optimization. In a game theoretic framework, the fitness of an individual strain depends on the strategies used by other strains—an outlook that is suited for describing both the between-host and the intracellular dynamics of multiple, competing phages. Inside a bacterial cell, phages can be thought of as agents with possibly incompatible mechanisms to exploit the cell. Outside a bacterial cell,

FIGURE 3



Basic framework of adaptive dynamics, where a resident sets the ecological equilibrium which is then subject to invasion by mutant types which may replace the residents. This framework will need to be adapted to the microbial world where evolutionary and ecological dynamics unfold on similar time scales.

direct interactions between phages are limited. As but one example of the utility of game theoretic thinking in the study of phages, intracellular cheating strains are favored when intracellular cooperative strains are common, even if those cheaters are worse off once they are alone. Such a result is indicative of the types of models necessary when considering the multitude of phages competing in realized environments.

Another formidable problem in the study of phages is how best to model the genotypic diversification of phages. One such approach, the quasi-species framework, assumes that a species figuratively is a “cloud,” consisting of many strains that contain mutationally linked genotypes. Sometimes a cloud of less-fit strains can outcompete an individual strain with higher fitness, particularly when the environment is highly variable. In addition, in those instances where long-term experiments have tracked viral adaptation, there is strong evidence of adapta-

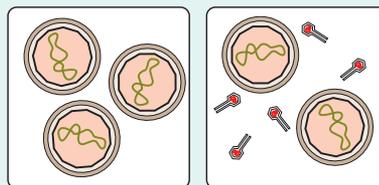


FIGURE 4

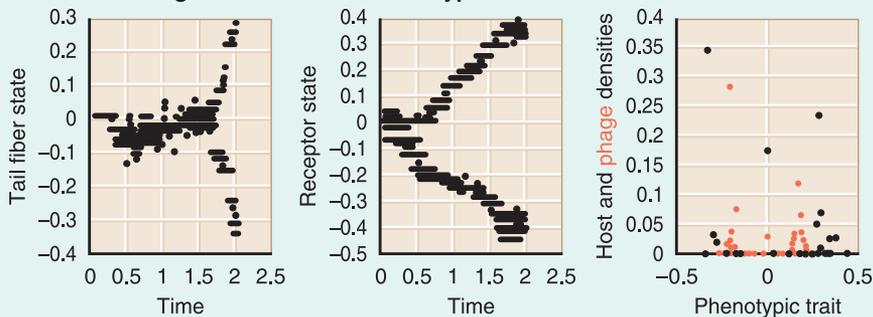
I. Population Dynamics of Resources (R), Bacteria (N), and Bacterial Viruses (V)

$$\begin{cases} \frac{dR}{dt} = -\omega(R - R_0) - \varepsilon\gamma(x) \frac{RN}{R + K} \\ \frac{dN}{dt} = -\omega N + \gamma(x) \frac{RN}{R + K} - \phi(x, y)NV \\ \frac{dV}{dt} = -\omega V + \beta\phi(x, y)NV \end{cases}$$

II. Ecological Outcomes: Phage Washout or Bacteria-Phage Coexistence



III. Coevolution-Induced Diversification: Multi-Strain Stochastic Simulations Reveal Emergence of Diverse Phenotypes



Coevolutionary dynamics of bacteria and bacteriophages are described using a phenotype-based population dynamics model (I), which leads to distinct ecological outcomes (II), and over time a hyper-diverse state (III). The bottom three figures denote the diversification of phage specificity, the diversification of growth rate efficiency of bacteria, and multi-strain coexistence in a homogeneous environment, respectively. More details concerning how the ecological model couples to trait changes and proofs of when diversification can occur are found in Weitz et al. (2005).

tion in genome substitution sequences. Such work suggests that variability of both the equilibrium state (arising from mutation selection balance) as well as adaptive trajectories can be addressed theoretically. However, we do not yet have general rules as to how fitness landscapes and mutational distributions are structured nor how they link to the within-host exploitation strategies discussed earlier.

Modeling Provides Insights about Host-Phage Coevolution

Pursuing a theoretical approach to studying phages eventually may help us to link data describing genetic diversity to changes in nutrient cycling and community organization at regional and even global scales. In the near term, improving models for connecting processes between closely linked scales (systems biology with physiology or ecology with evolution) are likely to

yield successes. Some recent theoretical work involving phages connects physiology, ecology, and evolutionary constraints. These vignettes illustrate ways in which theoretical approaches can provide insights about the evolution and ecology of bacterial viruses.

Phages are present in nearly every place where there is bacterial life on Earth, and are an incredibly diverse group of organisms. The driving forces behind such diversity are poorly understood and stand in contrast to numerous laboratory studies in which hosts quickly develop resistance to phage adsorption. In part to bridge this gap, my collaborators and I recently developed a computational framework for probing the driving mechanisms behind the diversity that can emerge from host-phage interactions. The multiscale model is based on the framework of adaptive dynamics (Fig. 3).

This framework starts with a population of interacting hosts and viruses, each group with fixed physiological traits. We then introduce mutations for both hosts and viruses, following diversity within this mixed population as it expands and contracts, responding to new traits and the changing fitness of the mutant strains. The dynamics include changes in population densities derived from hosts interacting with phages, and both host and phages adapting to new phenotypes, such as changes in growth rate of the hosts and in phage adsorptivity.

From these studies, we find that coevolution might act like a ratchet, enabling the host and phage to escape limits on diversity that a strictly ecological model would otherwise predict. We also find that coevolutionary branching occurs via a series of bifurcations (Fig. 4). For instance, when phage binding resembles that of an imperfect lock-key mechanism, there will be non-trivial outcomes of high-diversity assemblages of host and phage strains, a previously unrecognized finding. Diversity is facilitated when viruses are responsible for a large fraction of host

mortality, when virus host range is narrow (but not exclusive), and when viral mutants are introduced at a faster rate than that of hosts.

This mathematical model leaves out many complexities, but it points to sites in viral and bacterial genomes that may be under strong selection, including tail fiber genes in phage and membrane-bound nutrient uptake genes in bacteria, and ecological conditions that favor adaptive change.

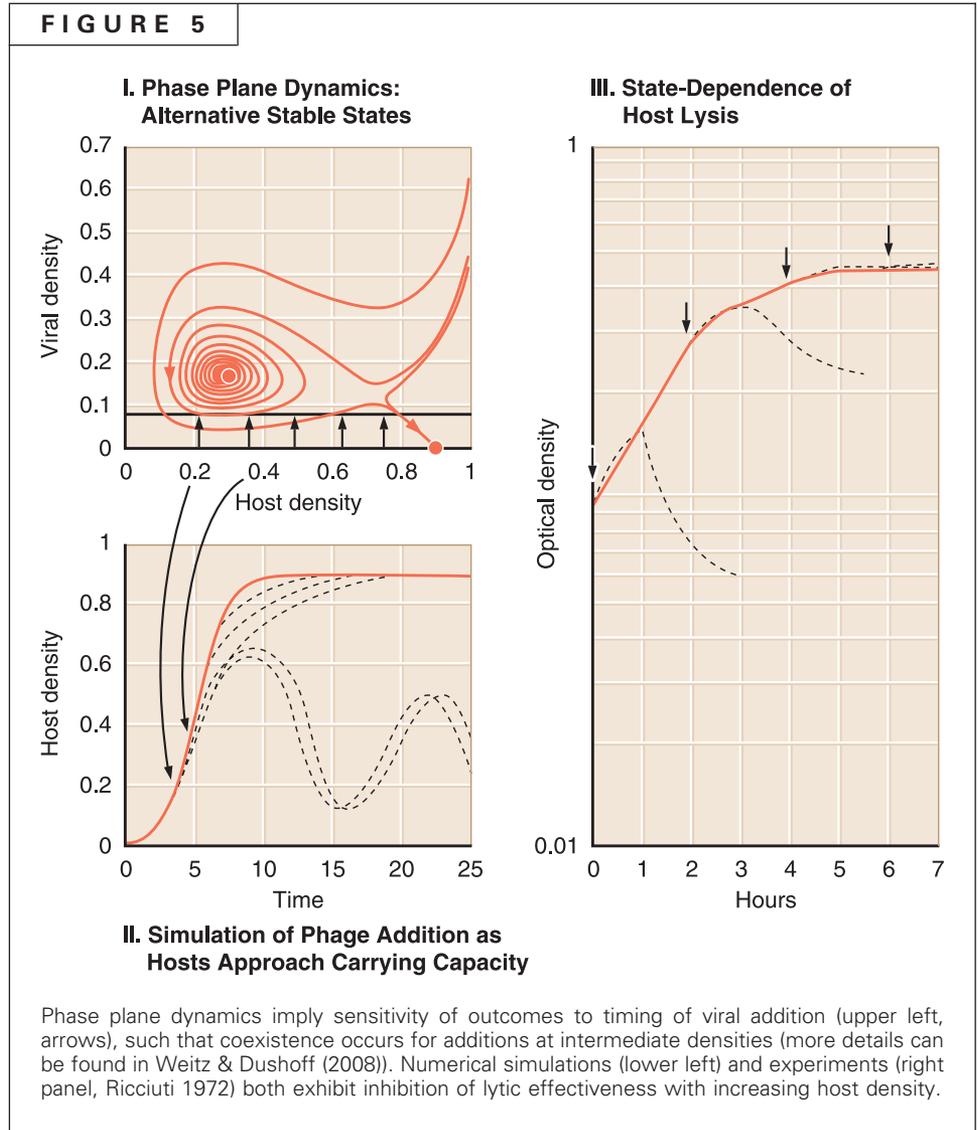
Modeling Provides Insights about Effects of Host Growth Stage on Phages

Although a simple model of host-phage interactions was introduced 30 years ago, little about many key aspects of within-host phage biology was integrated into that or other mathematical models of between-host dynamics. For example, whether grown in liquid culture or on bacterial lawns, the effectiveness of phages in lysing their hosts tends to decline as hosts approach the stationary phase of growth. To account for this lowering in host lysis rates as hosts approach carrying capacity, I recently led efforts to develop a simple, phenomenological model of host-phage population dynamics in which lysis rate depends on host density (Fig. 5).

The model adds the parameter a , the fractional reduction of lysis at carrying capacity, to an earlier model of host-phage dynamics. Thus, when $a = 0$, the model reduces to a simple analogue of the Levin-Stewart-Chao model of dynamics, whereas when $a = 1$, phages are completely inhibited at the host's carrying capacity. At intermediate values, lysis is partially reduced at carrying capacity. In contrast to underlying assumptions made by other models, phages cannot invade a host-only system at carrying capacity, but stably coexist with hosts at lower densities. This result is robust across large

changes in the value of a , meaning that even partial reduction of lysis near carrying capacity can have dramatic dynamic effects.

This model also predicts extreme sensitivity of outcomes to initial conditions. For example, in any particular host-phage mixture, the model predicts what range of phage densities will lead to host-phage coexistence. When too few phages are added, they cannot reproduce sufficiently to draw down hosts. However, if many more phage are introduced, they reproduce to produce a boom but subsequently crash, leading to their own extinction. The model also predicts that as hosts near saturation, the timing of viral additions can lead to radically different outcomes (Fig. 5). Ongoing work on the relation-





ship between host physiology and host-phage ecology includes investigations of a resource-explicit version of the model and an experimental search for alternative stable states.

More generally, phages exploit bacteria as part of a nested process that reflects distinct dynamics within and between hosts. Several intracellular processes govern the kinetic parameters that determine how a phage strain moves between hosts, including protein-protein interactions, regulatory controls, and timed events of

cell lysis. Although these processes are all subject to evolutionary changes, theoretical ecology is rooted in studies of comparatively slow-evolving organisms. Hence, a new theoretical foundation and new tools are needed if microbiologists are to translate evolutionary and ecological processes across multiple scales. Those theoretical tools are likely to provide key insights into the microbial and viral world, particularly if they are developed in complement with methods to directly access and assess high-throughput data.

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